EurJOC
European Journal of Organic Chemistry

Chemistry Europe
European Chemical Societies Publishing

Accepted Article

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To be cited as: *Eur. J. Org. Chem.* 10.1002/ejoc.202000684

Link to VoR: https://doi.org/10.1002/ejoc.202000684

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A Straightforward Synthesis of Trideuterated α-Terpinene for Mechanistic Studies

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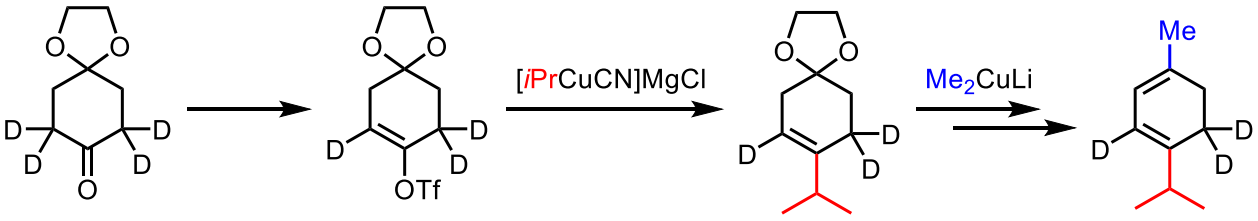
Abstract: Regiospecifically trideuterated (2,6,6-$^2H_3$)-α-terpinene was prepared in six steps and with a deuterium incorporation of >99% in 24% yield from 1,4-cyclohexanedione monoethylene ketal. The synthetic procedure involved twofold cross-coupling reactions of alkylcuprates (lithium dimethylcuprate and chloromagnesium cyano(isopropyl)cuprate, respectively) with enol triflates to introduce the alkyl substituents on the 1,3-cyclohexadiene backbone. By changing the alkylcuprates, the synthetic approach could serve as a prototype for the synthesis of various 1,4-dialkyl substituted 1,3-cyclohexadiene derivatives, which could be deuterium labeled as well, for example for mechanistic studies.



Graphical Abstract: Trideuterated (2,6,6-$^2H_3$)-α-terpinene was prepared in six steps from 1,4-cyclohexanedione monoethylene ketal. The key step of the synthesis was the twofold cross-coupling of alkylcuprates (lithium dimethylcuprate and chloromagnesium cyano(isopropyl)cuprate, respectively) with enol triflates.

Keywords: Hydrocarbons, Terpenes, Deuteration, Cross-coupling, Biodegradation

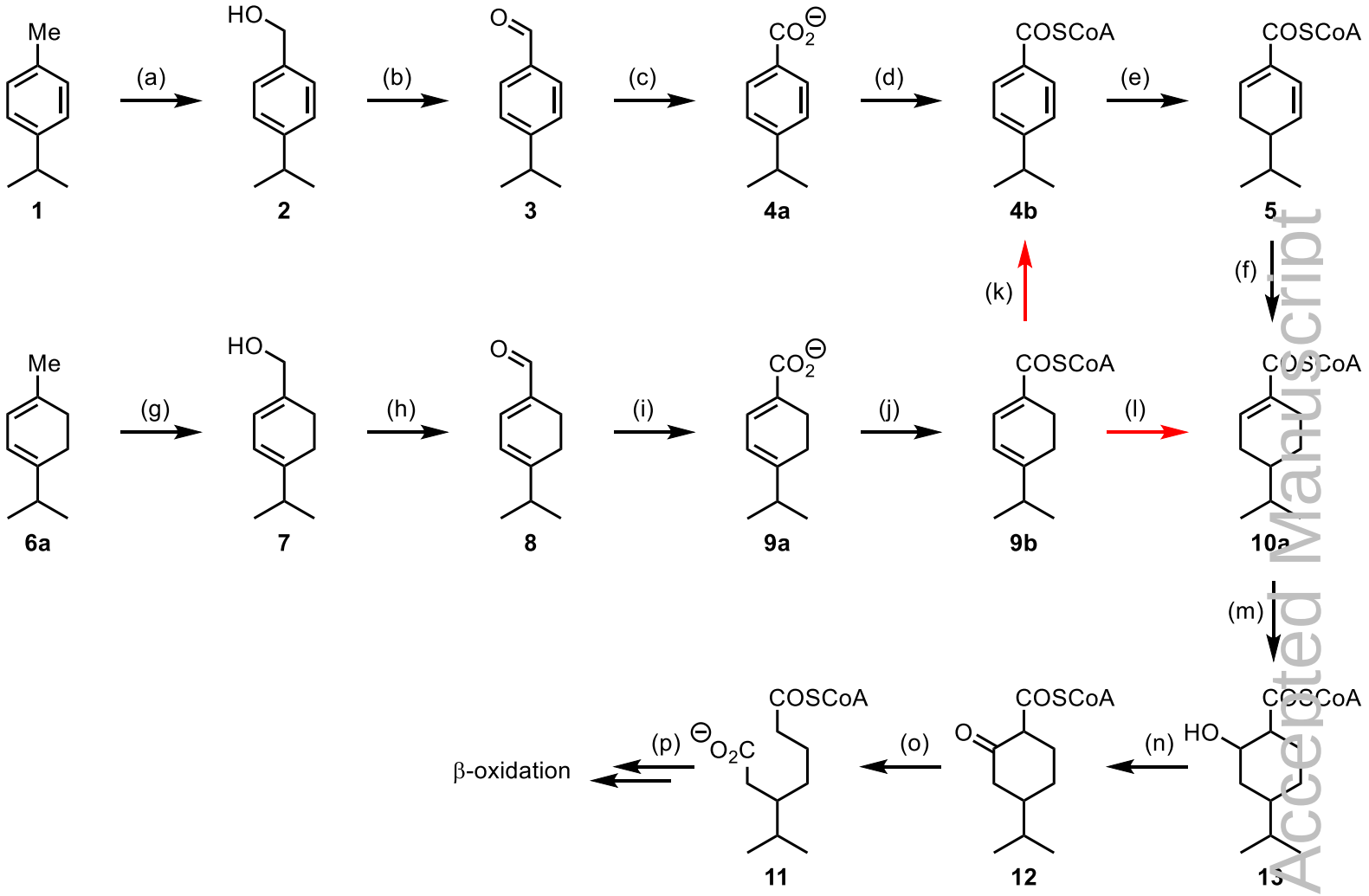
Introduction

The mechanisms of chemical and biological processes can be uniquely investigated using deuterium labeling, which enables the position specific incorporation of defined additional masses into an organic compound.[1] While the constitution of deuterated compounds is essentially the same, certain physico-chemical properties as well as the biological activities may change more or less significantly depending among others on the extent and the position of deuterium incorporation.[2–4] Today, deuterium labeling has many applications in nearly all disciplines in life sciences.[5] A prominent example is the deuterated drug Austedo (deutetrabenazine) for the treatment of Huntington's disease-related movement disorders, recently approved by the US Food and Drug Administration (FDA).[6]

Moreover, deuterium-labeled compounds are often used for the investigation of enzymatic reactions in biochemical pathways. Excellent reviews on various aspects of terpene biosynthesis have highlighted the utility of deuterium labeling in the elucidation of enzymatic reaction mechanisms.[7–9] For example, deuterium labeling experiments have been used to elucidate the biosynthetic pathway and establish *syn*-configuration of cyclic ether formation in the biosynthesis of the monoterpene 1,8-cineole (eucalyptol) in the herb *Salvia officinalis*[10] and the Gram-positive bacterium *Streptomyces clavuligerus*.[11] Likewise, *syn*-addition has been implicated in the mechanism of abiotic cyclization of nerol to eucalyptol inside a supramolecular catalyst on the basis of a deuterium-labeling experiment.[12] Other studies utilizing deuterium labeling have addressed open issues of sesquiterpene[13–17] and diterpene[18,19] biosynthesis. However, deuterium labeling also provides important insights into key reactions of biodegradation pathways. For example, the stereochemistry of the initial activation reaction of a hydrocarbon-transforming enzyme was previously elucidated using regio- and stereospecifically deuterium-labeled *n*-hexanes.[20] Also other examples, like the elucidation of the anaerobic degradation mechanism of triethanolamine by a homoacetogenic bacterium emphazise the importance of deuterium-labeled compounds for the characterization of biochemical pathways.[21]

Based on cultivation studies it has been shown that the denitrifying betaproteobacterium "*Aromatoleum aromaticum*" pCyN1 is capable to grow on different hydrocarbon substrates, such as aromatic (i.e. *p*-cymene, **1**) and non-aromatic (i.e. α-terpinene, **6a**) monoterpenes under anoxic conditions (Scheme 1).[22]

More recently, metabolite studies revealed that the pathway of the anaerobic degradation of *p*-cymene (**1**) starts with methyl-group hydroxylation (product **2**, Scheme 1, step a). After subsequent oxidation and thioesterification (steps b–d), the reductive dearomatization (step e), yielding dienoyl-CoA **5**, is catalyzed by a class I benzoyl-CoA reductase (BCR). After further functional group interconversions (steps f, m, n) the ring is cleaved to furnish 3-isopropylpimeloyl-CoA (**11**, step o).[23,24] Further investigations showed that the disproportionation of dienoyl-CoA **14** to benzoyl-CoA **16** and enoyl-CoA **15** is catalyzed by a tungsten-containing class II BCR under artificial conditions (Scheme 2, eq. 1) as well as by *Ferroglobus placidus* cell extracts by a so far unknown enzyme.[25,26] Latest investigations in our laboratories on methylated extracts of bacterial cultures of strain pCyN1 grown on α-terpinene (**6a**) led to the identification of four compounds, namely 4-isopropyl-1,5-cyclohexadiene-1-carbonyl-CoA (**9b**), 4-isopropyl-1-cyclohexene-1-carbonyl-CoA (**10a**), 2-hydroxy-4-isopropylcyclohexane-1-carbonyl-CoA (**13**) and 3-isopropylpimeloyl-CoA (**11**) (unpublished results; Rabus, Wilkes, Christoffers and co-workers). The presence of these compounds suggest a degradation pathway of α-terpinene (**6a**) that is similar to the known degradation pathway of *p*-cymene (**1**).[23,24] In addition, *p*-isopropylbenzoyl-CoA (**4b**) was abundant in the culture extracts (unpublished results; Rabus, Wilkes, Christoffers and co-workers), which is not necessarily expected to occur as an intermediate during anaerobic degradation of α-terpinene (**6a**). Based on these findings and taking into account the possibility of a disproportionation reaction, a pathway for the degradation of α-terpinene (**6a**) is proposed (Scheme 1, steps e–p), including a disproportionation of 4-isopropyl-1,3-cyclohexadiene-1-carbonyl-CoA (**9b**) to *p*-isopropylbenzoyl-CoA (**4b**) and 4-isopropyl-1-cyclohexene-1-carbonyl-CoA (**10a**) (Scheme 2, eq. 2). On the stage of these intermediates, the proposed pathway for the degradation of α-terpinene (**6a**) is supposed to converge with the pathway of *p*-cymene (**1**) degradation.



Scheme 1. Proposed pathways for the anaerobic degradation of *p*-cymene (**1**) and α-terpinene (**6a**) by strain pCyN1. The red arrows represent the proposed disproportionation reaction of dienoyl-CoA **9b** to *p*-isopropylbenzoyl-CoA (**4b**) and enoyl-CoA **10a**, converging with the pathway of *p*-cymene (**1**) degradation.

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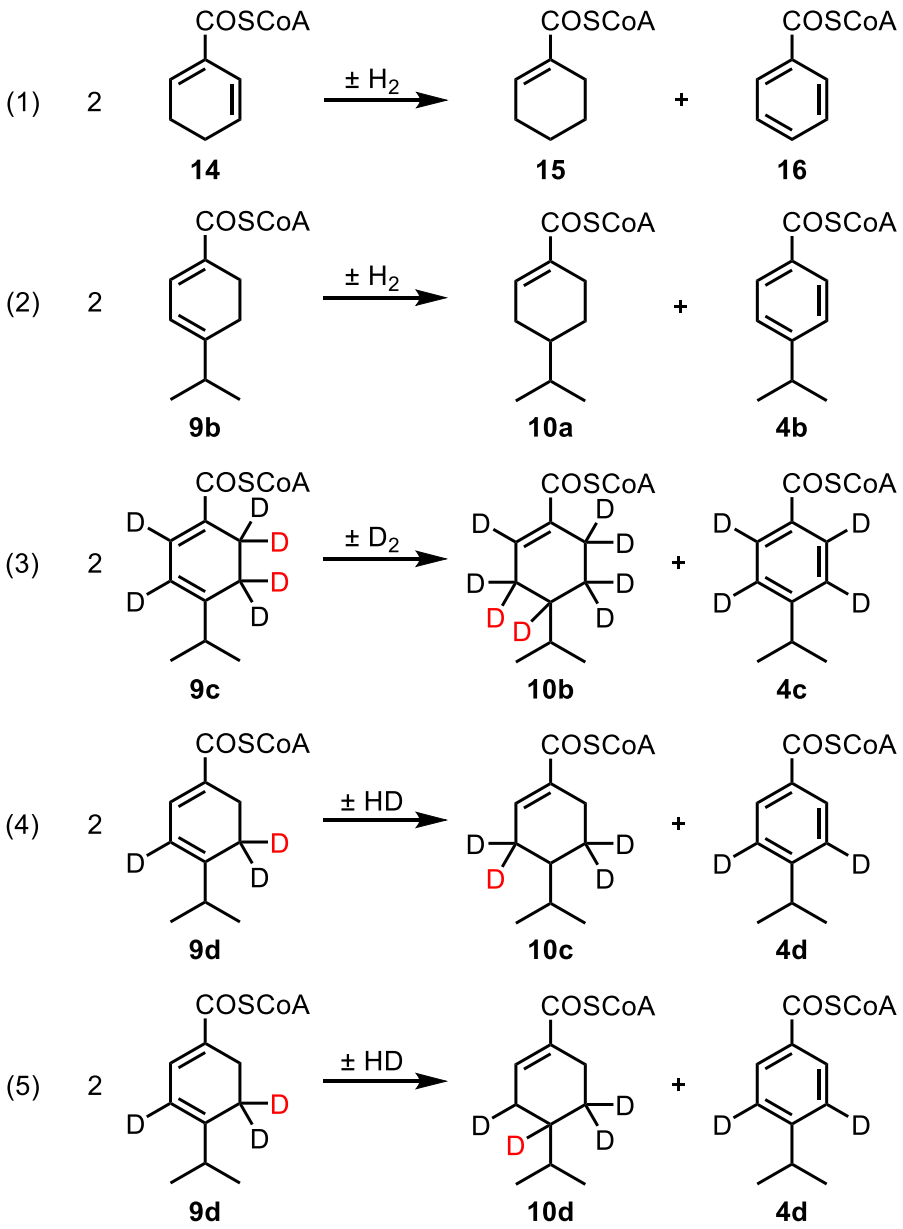

Scheme 2. Disproportionation of dienoyl-CoA **14** to enoyl-CoA **15** and benzoyl-CoA **16** (eq. 1), dienoyl-CoA **9b** to enoyl-CoA **10a** and 4-isopropylbenzoyl-CoA (**4b**) (eq. 2), dienoyl-CoA-d_6 **9c** to enoyl-CoA-d_8 **10b** and 4-isopropylbenzoyl-CoA-d_4 (**4c**) (eq. 3) and dienoyl-CoA-d_3 **9d** to 4-isopropylbenzoyl-CoA-d_2 (**4d**) and either enoyl-CoA-d_4 **10c** (eq. 4) or enoyl-CoA-d_4 **10d** (eq. 5).

As outlined above, the use of deuterated compounds could provide valuable insights into the reaction mechanisms of enzymes. Following this concept, we envisioned highly regiospecifically deuterated α-terpinene to be a perfect substrate for investigating the mechanisms of its degradation by the bacterial strain pCyN1. If the proposed disproportionation mechanism takes places in a classical manner, formally D_2 (Scheme 1, eq. 3) or HD (in case of eq. 4 and eq. 5) is abstracted from one molecule

of 4-isopropyl-1,3-cyclohexadiene-1-carbonyl-CoA (**9c** or **9d**) to introduce the double bond under formation of the aromatic system. The same two deuterium atoms (or one hydrogen atom and one deuterium atom in case of eq. 4 and eq. 5) are subsequently used for the hydrogenation of the double bond of a second molecule of 4-isopropyl-1,3-cyclohexadiene-1-carbonyl-CoA (**9c** or **9d**). The resulting, specifically deuterium-labeled, 4-isopropyl-1-cyclohexene-1-carbonyl-CoAs (**10b**, **10c** or **10d**) could be easily distinguished from a product that would not be formed by a disproportionation reaction according to their mass spectra using GC-MS after hydrolysis and methylation with diazomethane. The expected outcome of such an experiment would provide direct evidence for or against the proposed disproportionation even in whole cell experiments. However, it is clear that the elucidation of the molecular mechanism of this reaction would require access to the substrate enzyme complex.

A literature survey yielded only one reported access to deuterated α-terpinene, involving an ene reaction of liquid sulfur dioxide with α-terpinene (**6a**) accompanied with hydrogen–deuterium exchange at allylic carbon atoms.[27] However, we were not able to reproduce the reported results. Furthermore, considering the safety concerns and instrumental effort for performing reactions with liquid sulfur dioxide, we wished to have available a safer and easier access to a specifically deuterated α-terpinene. Therefore, the objective of this study was the development of a facile and reliable synthetic procedure to obtain regiospecifically deuterated α-terpinene.

Results and Discussion

The retrosynthetic analysis of deuterated α-terpinene is based upon twofold coupling of an alkylcuprate (methyl and isopropyl, respectively) and an enol triflate (**17** and **22**, respectively) as outlined in Scheme 3. Initially, we envisioned that starting the synthesis from 1,4-cyclohexanedione (**20a**) would be ideal since hydrogen–deuterium exchange in α-position of a carbonyl group is feasible by the α-acidity of this position. To protect the second carbonyl functionality during the first triflate formation and in the first cross-coupling reaction a 1,3-dioxolane would be a perfect choice. Since the isomer with conjugated double bonds should be thermodynamically favoured, it is very likely that the double bonds would arrange as the 1,3-diene **17** in the second triflate-forming reaction from compound **18**.

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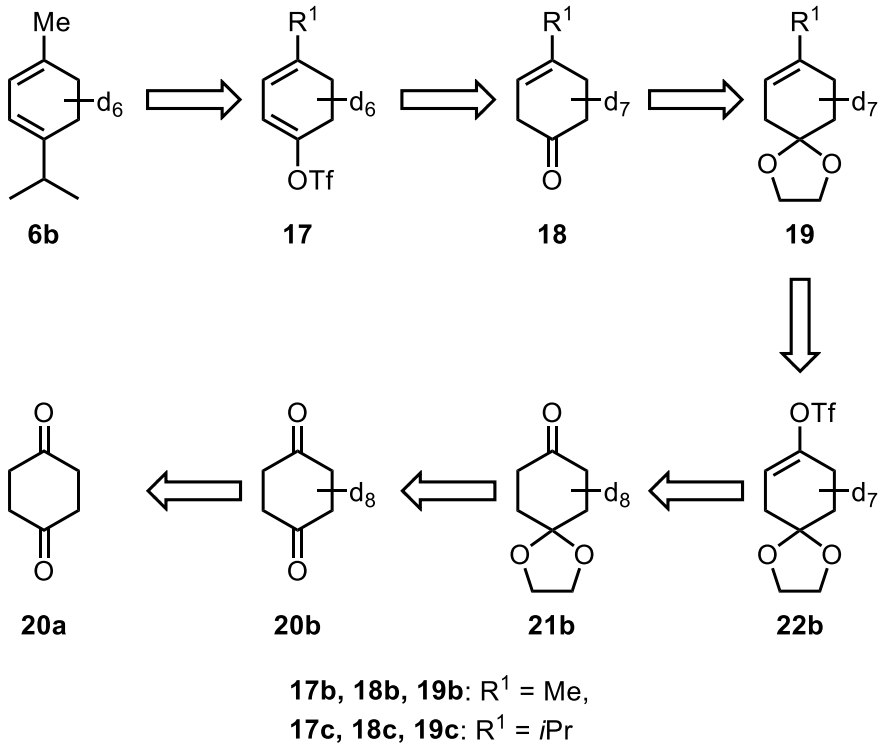
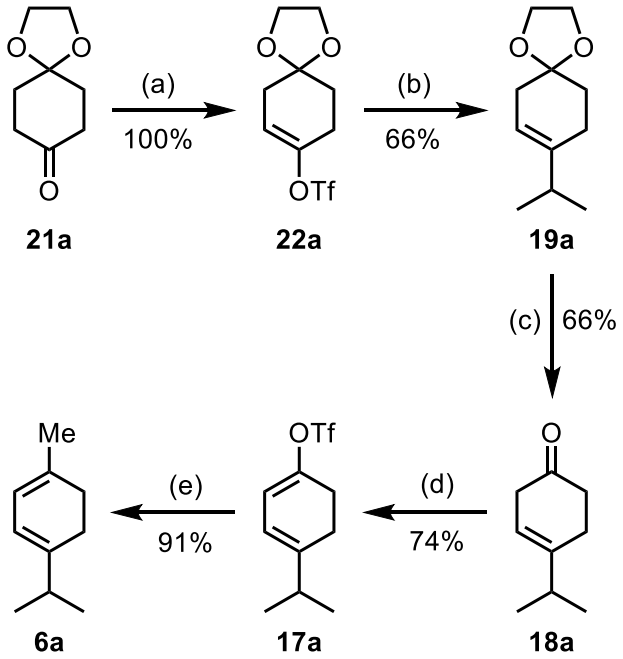


Scheme 3. Retrosynthetic analysis of hexadeuterated (2,3,5,5,6,6-$^2H_6$)-α-terpinene **6b**.

To proof our synthetic strategy we first carried it out without incorporation of deuterium, starting from commercially available 1,4-cyclohexanedione monoethylene ketal **21a** that was converted into the corresponding enol triflate **22a** using phenyl triflimide and LiHMDS (Scheme 4).[28,29] Our initial attempts included the introduction of the methyl group at first, followed by the introduction of the isopropyl group. Unfortunately, some resulting synthetic intermediates (the non-deuterated analogues of compounds **17b** and **18b**) turned out to be very volatile and relatively unstable. Therefore, we changed our strategy and introduced the isopropyl group in a cross-coupling reaction of the enol triflate **22a** with chloromagnesium cyano(isopropyl)cuprate first. Attempts using lithium diisopropylcuprate were less successful. The deprotection was performed with *p*TsOH×H_2O and the resulting β,γ-unsaturated ketone **18a** was converted directly without storage into the corresponding enol triflate **17a**, because the double bond of ketone **18a** readily isomerized to the α,β-conjugated system. After some experimentation, the reaction conditions for forming the enol triflate **17a** were optimized to shorter reaction times, which had the additional advantage that the previously observed formation of the aromatic side product by oxidation was significantly reduced. Finally, the cross-coupling of the enol triflate **17a** with lithium dimethylcuprate, applying

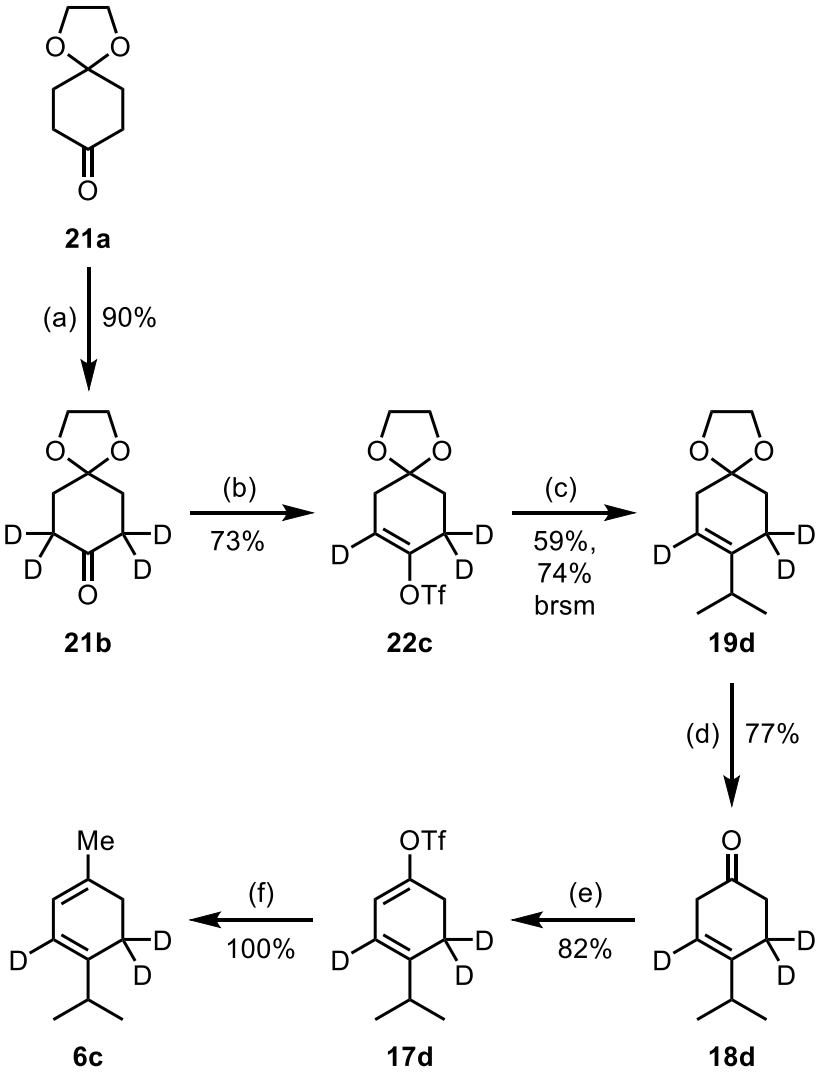
standard conditions[30] but changing the solvent to diethyl ether, furnished α-terpinene **6a** in excellent yield.



Scheme 4. Synthesis of non-deuterated α-terpinene **6a**. Reagents and conditions: (a) 1.1 equiv. LiHMDS, 1.1 equiv. $PhNTf_2$, THF, –78 to 23 °C, 14 h; (b) 8.5 equiv. *i*PrMgCl, 10 equiv. CuCN, Et_2O, –78 °C, 14 h; (c) 0.1 equiv. *p*TsOH×H_2O, acetone, 23 °C, 25 h; (d) 1.1 equiv. LiHMDS, 1.1 equiv. $PhNTf_2$, THF, –78 to 23 °C, 2 ¼ h; (e) 4.9 equiv. MeLi, 3.4 equiv. CuI, Et_2O, –15 °C, 14 h.

Next, attempts were made on the synthesis of a hexadeuterated (2,3,5,5,6,6-$^2H_6$)-α-terpinene (**6b**), starting with a literature-known deuteration of 1,4-cyclohexanedione (**20a**) using PCl_5 and D_2O.[31] The subsequent protection with ethylene glycol was performed following a slighty modified procedure, reported previously in the literature, including the four times repeated addition and removal of D_2O by azeotropic distillation from the reaction mixture.[32] As one could assume, the separation of the mono- and the diprotected 1,4-cyclohexanedione required a lot of effort. At this stage we initially recognized the loss of deuterium which was mainly due to column chromatography but also occurred upon storage. Therefore, we changed our strategy to the synthesis of a trideuterated (2,6,6-$^2H_3$)-α-terpinene (Scheme 5, **6c**), which should provide us the same information in a biological experiment (cf. Scheme 2, eq. 4 and eq. 5). As a great advantage, this opened the possibility to start with commercially available 1,4-cyclohexanedione monoethylene ketal **21a**. To avoid cleavage of the ketal, the deuteration reaction was changed to basic conditions using K_2CO_3, following a

modified procedure which has already been reported.[33] As outlined above, twofold cross-coupling reactions of the triflates **22c** and **17d** with chloromagnesium cyano(isopropyl)cuprate and lithium dimethylcuprate, respectively, furnished the targeted trideuterated α-terpinene **6c** with a deuterium incorporation >99%, according to NMR (no residual proton signals were detected at deuterated positions).



Scheme 5. Synthesis of trideuterated (2,6,6-$^{2}H_3$)-α-terpinene **6c**. Reagents and conditions: (a) 0.5 equiv. K_2CO_3, D_2O, 130 °C, 16 h, one repetition; (b) 1.1 equiv. LiHMDS, 1.1 equiv. $PhNTf_2$, THF, –78 to 23 °C, 5 h; (c) i: 3.2 equiv. *i*PrMgCl, 5.0 equiv. CuCN, Et_2O, –78 to 0 °C, 16 h; (d) 0.5 equiv. *p*TsOH×H_2O, acetone/H_2O 10:1, 23 °C, 23 h; (e) 1.1 equiv. LiHMDS, 1.1 equiv. $PhNTf_2$, THF, –78 °C to 23 °C, 3 h; (f) 6.1 equiv. MeLi, 3.6 equiv. CuI, Et_2O, –15 °C, 16 h.

Conclusion

In the course of our studies on the elucidation of anaerobic degradation pathways of monoterpenes in the denitrifying betaproteobacterium "*A. aromaticum*" pCyN1, we suggest the use of deuterated α-terpinene as a model substrate. Therefore, we developed a facile and reliable synthetic approach to the α-terpinene scaffold, which opens the possibility of deuterium incorporation. Following this approach, trideuterated (2,6,6-$^{2}H_3$)-α-terpinene **6c** was prepared in 24% yield (31% brsm) and with a deuterium incorporation >99% over six steps starting from 1,4-cyclohexanedione monoethylene ketal **21a**. The synthetic procedure involved twofold cross-coupling reactions of dialkylcuprates or alkylcyanocuprates with the enol triflates **22c** and **17d** to introduce the alkyl substituents of the 1,3-cyclohexadiene backbone. In general, this approach offers the possibility to adopt the synthetic procedure in the synthesis of various 1,4-dialkylsubstituted 1,3-cyclohexadiene derivatives by changing the corresponding alkylcuprates. In particular, these derivatives could be as well synthesized with a highly and well-defined deuterium incorporation, using them for example in mechanistic studies.

Experimental Section

General: Unless otherwise noted, all synthetic transformations were performed under inert conditions (nitrogen atmosphere, exclusion of air and moisture) using anhydrous solvents. Preparative column chromatography was carried out using Merck SiO_2 (40–63 µm, type 60 A) with hexanes (mixture of isomers, bp. 64–71 °C), pentane, *tert*-butyl methyl ether (MTBE) and diethyl ether (Et_2O) as eluents. TLC was performed on aluminum plates coated with SiO_2 F_{254}. GC-MS analyses were performed on a Shimadzu GCMS-QP2020 using a Macherey-Nagel OPTIMA 5 HT fused silica capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness). Helium was used as carrier gas. The GC oven temperature was programmed from 50 °C (2 min hold time) to 250 °C at a rate of 30 K min^{-1} (7 min hold time). The MS was operated in EI mode (70 eV) at a source temperature of 230 °C and a transfer line temperature of 280 °C. The mass range was 50–600 Dalton at a scan cycle time of 0.2 s. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 300 instrument. Multiplicities of carbon signals were determined with DEPT experiments. HRMS spectra of products were obtained with a Waters Q-TOF Premier (ESI) or Thermo Scientific DFS (EI) spectrometer. IR spectra were recorded on a Shimadzu IRSpirit

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spectrometer equipped with a diamond ATR unit. All starting materials were commercially available.

1,4-Dioxaspiro[4.5]dec-7-en-8-yl trifluoromethanesulfonate (22a). According to a slightly modified procedure reported previously in the literature,[28] LiHMDS (1.3 mol/L solution in THF, 16.3 mL, 21.1 mmol, 1.1 equiv.) was added dropwise over a period of 15 min to a solution of ketone **21a** (3.00 g, 19.2 mmol) and $PhNTf_2$ (7.55 g, 21.1 mmol, 1.1 equiv.) in THF (80 mL) at –78 °C. After stirring for 1 h at this temperature, the reaction mixture was warmed to ambient temperature and stirring was continued for 13 h. The reaction mixture was diluted with H_2O (75 mL), extracted with MTBE (3 × 100 mL), washed with H_2O (250 mL) and brine (250 mL), dried over $MgSO_4$ and filtered. The solvent was evaporated to yield the title compound **22a** as a slightly yellowish liquid (5.54 g, 19.2 mmol, 100%) which was used without further purification in the next step. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.90 (t, *J* = 6.5 Hz, 2H), 2.40–2.41 (m, 2H), 2.52–2.56 (m, 2H), 3.99 (s, 4H), 5.65–5.67 (m, 1H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ = 26.54 (CH_2), 31.16 (CH_2), 34.31 (CH_2), 64.84 (2 × CH_2), 106.28 (C), 115.95 (CH), 118.66 (q, *J* = 318.3 Hz, CF_3), 148.33 (C) ppm. All data were in accordance with the literature.[34]

8-Isopropyl-1,4-dioxaspiro[4.5]dec-7-ene (19a). According to a slightly modified procedure reported previously in the literature,[35] *i*PrMgCl (2.0 mol/L solution in THF, 47.2 mL, 94.4 mmol, 8.5 equiv.) was added dropwise over a period of 35 min to a suspension of CuCN (9.94 g, 111 mmol, 10 equiv.) in Et_2O (100 mL) at –78 °C. The reaction mixture was warmed to 0 °C, stirred for 15 min at this temperature and cooled again to –78 °C. Then, a solution of triflate **22a** (3.20 g, 11.1 mmol) in 50 mL Et_2O was added dropwise over a period of 40 min. After stirring at –78 °C for 14 h the reaction mixture was diluted with saturated, aqueous NH_4Cl solution (150 mL) and filtered through a plug of SiO_2 (2 cm, rinsed with MTBE). The organic layer was separated and the aqueous layer extracted with MTBE (3 × 150 mL). The combined organic layers were washed with H_2O (150 mL) and brine (150 mL), dried over $MgSO_4$, filtered and evaporated. The crude product was submitted to column chromatography (SiO_2, hexanes/MTBE, 9:1, R_f = 0.28) to furnish the title compound **19a** (1.34 g, 7.34 mmol, 66%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.01 (d, *J* = 6.8 Hz, 6H), 1.75 (t, *J* = 6.5 Hz, 2H), 2.17–2.26 (m, 5H), 3.98 (s, 4H), 5.28–5.33 (m, 1H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ = 21.52 (2 × CH_3), 25.38 (CH_2), 31.49 (CH_2), 34.66

(CH), 35.82 (CH_2), 64.52 (2 × CH_2), 108.45 (C), 115.71 (CH), 143.20 (C) ppm. All data were in accordance with the literature.[36]

4-Isopropyl-3-cyclohexen-1-one (18a). *p*TsOH×H_2O (117 mg, 0.615 mmol, 0.1 equiv.) was added to a solution of ketal **19a** (1.12 g, 6.14 mmol) in acetone (20 mL). After stirring for 27 h at ambient temperature in the dark, $NaHCO_3$ (7.40 g, 88.1 mmol) was added, the resulting suspension was filtered (rinsed with Et_2O) and evaporated. The crude product was submitted to column chromatography (SiO_2, pentane/Et_2O, 4:1, R_f = 0.48) to furnish the title compound **18a** (557 mg, 4.03 mmol, 66%, based on 88% purity established by ^{1}H NMR) as a colorless liquid. The title compound was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.03 (d, *J* = 6.8 Hz, 6H), 2.26–2.50 (m, 5H), 2.83–2.87 (m, 2H), 5.44–5.46 (m, 1H) ppm. ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): δ = 21.28 (2 × CH_3), 26.47 (CH_2), 34.88 (CH), 39.04 (CH_2), 39.78 (CH_2), 115.59 (CH), 144.74 (C), 211.66 (C) ppm. All data were in accordance with the literature.[37]

4-Isopropyl-1,3-cyclohexadiene-1-yl trifluoromethanesulfonate (17a). According to a slightly modified procedure reported previously in the literature,[28] LiHMDS (1.3 mol/L solution in THF, 3.8 mL, 4.9 mmol, 1.1 equiv.) was added dropwise over a period of 15 min to a solution of ketone **18a** (614 mg, 4.44 mmol) and $PhNTf_2$ (1.75 g, 4.89 mmol, 1.1 equiv.) in THF (30 mL) at –78 °C. After stirring for 15 min at this temperature, the reaction mixture was warmed to ambient temperature and stirring was continued for 2 h. The reaction mixture was diluted with H_2O (25 mL), extracted with Et_2O (3 × 30 mL), washed with H_2O and brine (both 50 mL), dried over $MgSO_4$, filtered and evaporated. The crude product was submitted to column chromatography (SiO_2, pentane, R_f = 0.21) to furnish the title compound **17a** (889 mg, 3.29 mmol, 74%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.04 (d, *J* = 6.8 Hz, 6H), 2.26–2.41 (m, 3H), 2.50–2.56 (m, 2H), 5.57 (d, *J* = 6.1 Hz, 1H), 5.88 (d, *J* = 6.2 Hz, 1H) ppm. ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): δ = 21.10 (2 × CH_3), 26.39 (CH_2), 26.53 (CH_2), 34.47 (CH), 113.59 (CH), 115.47 (CH), 118.74 (q, *J* = 318.5 Hz, CF_3), 146.34 (C), 146.86 (C) ppm. IR (ATR): $\tilde{\nu}$ = 2966 (w), 2877 (w), 2836 (w), 1670 (w), 1607 (w), 1467 (w), 1417 (s), 1386 (w), 1369 (w), 1247 (m), 1203 (vs), 1139 (s), 1082 (s), 1051 (w), 1013 (w), 979 (m), 879 (s), 853 (s), 833 (s), 774 (w), 763 (w), 746 (w), 677 (w), 611 (s), 510 (m) cm^{-1}. HRMS (EI): calcd. 270.0532 (for $C_{10}H_{13}F_3O_3S^+$), found 270.0527 [M^+]; $C_{10}H_{13}F_3O_3S$

(270.27). GC-MS (EI, 70 eV): *m/z* (%) 270 (18) [M^+], 255 (3), 137 (17), 121 (6), 109 (20), 95 (100), 93 (10), 91 (10), 81 (18), 79 (15), 77 (15), 69 (23), 67 (27), 65 (10), 55 (25), 53 (13).

α-Terpinene (6a). According to a procedure reported previously in the literature,[30] MeLi (1.6 mol/L solution in Et_2O, 2.3 mL, 3.7 mmol, 5.0 equiv.) was added dropwise over a period of 5 min to a suspension of CuI (480 mg, 2.52 mmol, 3.4 equiv) in 5 mL Et_2O at 0 °C. After stirring for 5 min, a solution of triflate **17a** (200 mg, 0.74 mmol) in 3 mL Et_2O was added dropwise over a period of 5 min at 0 °C, followed by stirring for 14 h at –15 °C. The reaction mixture was diluted with pentane (5 mL), filtered through a plug of SiO_2 (2 cm, rinsed with pentane) and evaporated. The crude product was submitted to column chromatography (SiO_2 impregnated with 8 wt% $AgNO_3$,[38,39] pentane) to furnish the title compound **6a** (102 mg, 0.75 mmol, quant., based on 89% purity established by ^{1}H NMR) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.03 (d, *J* = 6.8 Hz, 6H), 1.77 (s, 3H), 2.03–2.17 (m, 4H), 2.28 (hept, *J* = 6.8 Hz, 1H), 5.58–5.64 (m, 2H) ppm. ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): δ = 21.35 (2 × CH_3), 23.09 (CH_3), 25.42 (CH_2), 29.14 (CH_2), 34.64 (CH), 116.50 (CH), 119.61 (CH), 133.37 (C), 142.68 (C) ppm. ^{13}C Data were in accordance with the literature.[40]

(7,7,9,9-$^2H_4$)-1,4-Dioxaspiro[4.5]decan-8-one (21b). According to a slightly modified procedure reported previously in the literature,[33] K_2CO_3 (1.33 g, 9.61 mmol, 0.5 equiv.) was added to a solution of 1,4-dioxaspiro[4.5]decan-8-one (**21a**, 3.00 g, 19.2 mmol) in D_2O (25 mL) and the mixture was stirred at 130 °C over night. The mixture was extracted with abs. Et_2O (4 × 25 mL), dried over $MgSO_4$, filtered and evaporated. The procedure described above was repeated once to furnish the title compound **21b** (2.77 g, 17.3 mmol, 90%) as a slightly brownish solid which was used in the next step without further purification. Mp. = 71–72 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.00 (s, 4H), 4.03 (s, 4H) ppm. ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): δ = 33.77 (2 × CH_2), 37.57 (pent, *J* = 19.9 Hz, 2 × C^2H_2), 64.66 (2 × CH_2), 107.14 (C), 210.61 (C) ppm. IR (ATR): $\tilde{\nu}$ = 2959 (w), 2886 (m), 2227 (w), 1706 (vs), 1476 (w), 1436 (m), 1357 (w), 1342 (w), 1277 (m), 1226 (w), 1206 (w), 1177 (m), 1124 (m), 1070 (s), 1051 (m), 1029 (m), 1009 (s), 974 (w), 947 (m), 891 (w), 841 (s), 769 (w), 759 (w), 713 (m), 643 (m) cm^{-1}. HRMS (ESI, pos.

mode): calcd. 167.1192 (for $C_8H_8{}^2H_4LiO_3^+$), found 167.1195 [M + Li^+]; $C_8H_8{}^2H_4O_3$ (160.21). GC-MS (EI, 70 eV): *m/z* (%) 160 (2) [M^+], 102 (41), 101 (100), 100 (20), 86 (7), 71 (4), 58 (19), 57 (20), 56 (10).

(7,7,9-$^2H_3$)-1,4-Dioxaspiro[4.5]dec-7-en-8-yl trifluoromethanesulfonate (22c). According to the procedure reported above for the non-deuterated compound **22a**, LiHMDS (1.3 mol/L solution in THF, 13.2 mL, 17.2 mmol, 1.1 equiv.), ketone **21b** (2.50 g, 15.6 mmol) and $PhNTf_2$ (6.13 g, 17.2 mmol, 1.1 equiv.) were converted in THF (70 mL) to furnish the title compound **22c** after column chromatography (SiO_2, hexanes/MTBE, 3:2, R_f = 0.32) as a slightly yellowish liquid (3.34 g, 11.5 mmol, 73%). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.89 (s, 2H), 2.40 (s, 2H), 3.98 (s, 4H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ = 25.88 (pent, *J* = 20.0 Hz, C^2H_2), 30.96 (CH_2), 34.17 (CH_2), 64.82 (2 × CH_2), 106.25 (C), 115.79 (t, *J* = 24.0 Hz, C^2H), 118.63 (q, *J* = 318.2 Hz, CF_3), 148.20 (C) ppm. IR (ATR): $\tilde{\nu}$ = 2966 (w), 2889 (w), 1676 (w), 1414 (s), 1362 (w), 1344 (w), 1309 (w), 1282 (w), 1247 (m), 1202 (vs), 1137 (s), 1126 (s), 1069 (s), 1036 (s), 999 (s), 947 (m), 870 (s), 853 (m), 836 (s), 810 (s), 769 (w), 743 (w), 723 (w), 701 (w), 677 (w), 659 (w), 609 (s), 570 (m), 517 (m) cm^{-1}. HRMS (ESI, pos. mode): calcd. 314.0360 (for $C_9H_8{}^2H_3F_3NaO_5S^+$), found 314.0353 [M + Na^+], $C_9H_8{}^2H_3F_3O_5S$ (291.26). GC-MS (EI, 70 eV): *m/z* (%) 159 (9), 158 (100), 142 (2), 130 (8), 128 (7), 114 (22), 101 (5), 86 (39), 74 (9), 70 (14), 69 (23), 58 (16), 57 (9), 56 (58).

(7,7,9-$^2H_3$)-8-Isopropyl-1,4-dioxaspiro[4.5]dec-7-ene (19d). According to the procedure reported above for the non-deuterated compound **19a**, *i*PrMgCl (2.0 mol/L solution in THF, 22.0 mL, 43.9 mmol, 3.2 equiv.), CuCN (6.15 g, 68.7 mmol, 5 equiv.) and triflate **22c** (4.00 g, 13.7 mmol) were converted in Et_2O (100 mL) to furnish the title compound **19d** (1.51 g, 8.14 mmol, 59%, 74% brsm) after column chromatography (SiO_2, hexanes/MTBE, 9:1 to 1:1, R_f = 0.22) as a colorless liquid. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.01 (d, *J* = 6.9 Hz, 6H), 1.74 (s, 2H), 2.21 (hept, *J* = 6.9 Hz, 1H), 2.25 (s, 2H), 3.98 (s, 4H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ = 21.37 (2 × CH_3), 24.55 (pent, *J* = 19.2 Hz, C^2H_2), 31.20 (CH_2), 34.47 (CH), 35.58 (CH_2), 64.38 (2 × CH_2), 108.31 (C), 115.37 (t, *J* = 23.5 Hz, C^2H), 142.83 (C) ppm. IR (ATR): $\tilde{\nu}$ = 2957 (m), 2927 (w), 2873 (m), 2252 (w), 2190 (w), 2094 (w), 1467 (w), 1424 (w), 1360 (m), 1342 (w), 1306 (w),

1273 (w), 1253 (w), 1124 (s), 1060 (s), 1036 (m), 994 (w), 964 (w), 946 (m), 861 (m), 841 (m), 814 (m), 761 (w), 661 (w), 533 (w) cm^{-1}. HRMS (EI): calcd. 185.1490 (for $C_{11}H_{15}{}^{2}H_3O_2^+$), found 185.1491 [$M^+$]; $C_{11}H_{15}{}^{2}H_3O_2$ (185.28). GC-MS (EI, 70 eV): *m/z* (%) 185 (16) [M^+], 170 (8), 155 (2), 142 (3), 140 (3); 126 (2), 99 (3), 98 (3), 87 (9), 86 (100), 68 (3), 57 (3), 56 (3).

(3,5,5-$^{2}H_3$)-4-Isopropyl-3-cyclohexen-1-one (18d). According to the procedure reported above for the non-deuterated compound **18a**, *p*TsOH×H_2O (590 mg, 3.11 mmol, 0.5 equiv.) and ketal **19d** (1.15 g, 6.21 mmol) were converted in a mixture of acetone (20 mL) and H_2O (2 mL) to furnish the title compound **18d** (678 mg, 4.80 mmol, 77%, based on 91% purity established by ^{1}H NMR) after column chromatography (SiO_2, pentane/Et_2O, 2:1, R_f = 0.39) as a colorless liquid. The title compound was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.04 (d, *J* = 6.9 Hz, 6H), 2.31 (hept, *J* = 6.9 Hz, 1H), 2.46 (s, 2H), 2.85 (s, 2H) ppm. $^{13}C\{^{1}H\}$ NMR (75 MHz, $CDCl_3$): δ = 21.06 (2 × CH_3), 25.61 (pent, *J* = 19.5 Hz, $C^{2}H_2$), 34.61 (CH), 38.65 (CH_2), 39.45 (CH_2), 115.17 (t, *J* = 23.9 Hz, $C^{2}H$), 144.30 (C), 211.29 (C) ppm. IR (ATR): $\tilde{\nu}$ = 2960 (m), 2932 (w), 2872 (w), 1717 (vs), 1467 (w), 1404 (w), 1383 (w), 1363 (w), 1292 (w), 1269 (w), 1252 (w), 1233 (w), 1203 (w), 1124 (w), 1099 (w), 1074 (w), 1064 (w), 1031 (w), 929 (w), 881 (w), 861 (w), 837 (w), 819 (w), 700 (w), 687 (w), 669 (w), 523 (w) cm^{-1}. HRMS (EI): calcd. 141.1227 (for $C_9H_{11}{}^{2}H_3O^+$), found 141.1220 [M^+]; $C_9H_{11}{}^{2}H_3O$ (141.23). GC-MS (EI, 70 eV): *m/z* (%) 141 (43) [M^+], 126 (5), 111 (2), 99 (35), 98 (19), 84 (100), 83 (36), 82 (29), 70 (13), 69 (14), 68 (16), 67 (7), 57 (12), 56 (14), 55 (10).

(3,5,5-$^2H_3$)-4-Isopropyl-1,3-cyclohexadiene-1-yl trifluoromethanesulfonate (17d). According to the procedure reported above for the non-deuterated compound **17a**, LiHMDS (1.3 mol/L solution in THF, 2.10 mL, 2.73 mmol, 1.1 equiv.), ketone **18d** (350 mg, 2.48 mmol) and $PhNTf_2$ (975 mg, 2.73 mmol, 1.1 equiv.) were converted in THF (15 mL) to furnish the title compound **17d** (558 mg, 2.04 mmol, 82%, based on 95% purity established by ^1H NMR) after column chromatography (SiO_2, pentane, R_f = 0.24) as a colorless liquid. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.04 (d, *J* = 6.8 Hz, 6H), 2.32 (hept, *J* = 6.9 Hz, 1H), 2.51 (s, 2H), 5.88 (s, 1H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ = 21.02 (2 × CH_3), 25.79 (pent, *J* = 19.6 Hz, C^2H_2), 26.19 (CH_2), 34.39 (CH), 113.33 (t, *J* = 24.5 Hz, C^2H), 115.37 (CH), 118.74 (q, *J* = 318.6 Hz, CF_3), 146.10 (C), 146.89 (C) ppm. IR (ATR): $\tilde{\nu}$ = 2966 (w), 2876 (w), 1659 (w), 1467 (w), 1417 (s), 1386 (w), 1367 (w), 1354 (w), 1299 (w), 1246 (m), 1202 (vs), 1139 (s), 1090 (s), 1070 (w), 1056 (m), 1036 (w), 963 (m), 893 (s), 877 (m), 841 (s), 821 (s), 769 (w), 737 (w), 719 (w), 683 (w), 663 (w), 609 (s), 580 (w), 561 (w), 507 (m) cm^{-1}. HRMS (EI): calcd. 273.0720 (for $C_{10}H_{10}{}^2H_3F_3O_3S^+$), found 273.0719 [$M^+$]; $C_{10}H_{10}{}^2H_3F_3O_3S$ (273.28). GC-MS (EI, 70 eV): *m/z* (%) 273 (20) [M^+], 140 (22), 123 (7), 112 (22), 98 (100), 97 (22), 96 (17), 83 (15), 82 (13), 70 (15), 69 (40), 68 (11), 57 (16), 56 (11), 55 (11).

(2,6,6-$^2H_3$)-α-Terpinene (6c). According to the procedure reported above for the non-deuterated compound, MeLi (1.6 mol/L solution in Et_2O, 30.7 mL, 49.1 mmol, 6.1 equiv.), CuI (5.54 g, 29.1 mmol, 3.6 equiv.) and triflate **17d** (2.20 g, 8.06 mmol) were converted in Et_2O (75 mL) to furnish the title compound **6c** (1.14 g, 8.19 mmol, quant.) after column chromatography (SiO_2, pentane, R_f = 0.51) as a colorless liquid. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.02 (d, *J* = 6.9 Hz, 6H), 1.77 (s, 3H), 2.07 (s, 2H), 2.28 (hept, *J* = 6.9 Hz, 1H), 5.62 (s, 1H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ = 21.31 (2 × CH_3), 23.07 (CH_3), 24.61 (pent, *J* = 19.1 Hz, C^2H_2), 28.92 (CH_2), 34.54 (CH), 116.24 (t, *J* = 23.6 Hz, C^2H), 119.52 (CH), 133.25 (C), 142.35 (C) ppm. IR (ATR): $\tilde{\nu}$ = 3030 (w), 2959 (m), 2922 (w), 2870 (w), 2819 (w), 2266 (w), 2247 (w), 2186 (w), 2084 (w), 1646 (w), 1447 (w), 1429 (w), 1380 (w), 1362 (w), 1303 (w), 1260 (w), 1134 (w), 1096 (w), 1074 (w), 1030 (w), 977 (w), 947 (w), 874 (w), 854 (w), 841 (w), 806 (w), 709 (w), 680 (w), 661 (w), 654 (w), 560 (w), 550 (w), 510 (w) cm^{-1}. HRMS (EI): calcd. 139.1435 (for $C_{10}H_{13}{}^2H_3^+$), found 139.1435 [M^+]; $C_{10}H_{13}{}^2H_3$ (139.26). GC-MS (EI, 70 eV): *m/z* (%)

139 (49) [M^+], 124 (100), 110 (8), 109 (12), 108 (12), 96 (52), 95 (38), 94 (30), 93 (23), 82 (14), 81 (17), 80 (15), 79 (18), 78 (12), 67 (5), 66 (5), 52 (6).

Acknowledgment

This work was generously supported by the Deutsche Forschungsgemeinschaft (GRK 2226). MTBE was obtained as a generous gift from Evonik Industries, Marl, Germany.

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